

ACTA BOTANICA FENNICA 36  
EDIDIT  
SOCIETAS PRO FAUNA ET FLORA FENNICA

SOME EXPERIMENTS ON THE EXCHANGE  
OF POTASSIUM AND SODIUM BETWEEN  
SINGLE CELLS OF CHARACEAE AND THE  
BATHING FLUID

BY  
IB HOLM-JENSEN, AUGUST KROGH  
AND  
VEIJO WARTIOVAARA

FROM THE LABORATORY OF ZOOPHYSIOLOGY, COPENHAGEN UNIVERSITY

HELSINGFORSIAE 1944

PRINTED BY  
OY TILGMANN AB, HELSINKI 1944

## Introduction.

The experiments to be described in the following were undertaken on the joint initiative of professor R. COLLANDER and A. K. as a continuation of the work of COLLANDER and collaborators on permeability problems in *Characeae*. As they could only be made by means of radioactive isotopes it was decided to carry them out in Copenhagen and to utilize the cyclotron of the Institute for Theoretical Physics to obtain the active preparations<sup>1)</sup>. Dr. WARTIOVAARA came to Copenhagen in Sept. 1942 and brought with him a supply of *Tolypellopsis*, but unfortunately the cyclotron failed at the time, and the experiments desired could not be made during W.s stay. However, so much was done and the technique was so far perfected by him that he should be counted one of the authors, although he could not take part in the determinations finally carried out in the winter of 1943/44. They were begun in the autumn, but had to be suspended during December and January owing to the seizure of the Institute for Theoretical Physics. When they could again be made the cell material available was rather limited and was supplemented with cells of *Nitella* obtained from the Botanical Institute of Stockholms Högskola by the kindness of professor STÅLFELT.

COLLANDER (1939) showed by spectrographic analysis of the cell sap and outside solution in different experimental conditions that the permeability of *Chara* and *Tolypellopsis* cells for kations is very low, 1 millionth or less of the amount which would diffuse through a corresponding layer of water (5 $\mu$  thick), but his results were complicated by the active ion absorption going on, and it was impossible to distinguish with any certainty between these two processes. COLLANDER found for instance that lithium would be absorbed by *Chara* cells from an otherwise normal bathing fluid, containing 1 mM lithium, so that the Li-concentration in the sap would exceed the outside after two days and go on increasing for at least a fortnight to more than 5 mM. When such cells were put into a Li-free solution no measurable amount of Li would come out during two days. There can be little doubt that in this case the Li uptake was an active process.

---

<sup>1)</sup> We are extremely grateful for the kindness shown us by the personnel working the cyclotron who placed extremely active preparations of <sup>24</sup>Na and <sup>42</sup>K at our disposal.

When radioactive isotopes are used we have to do with atoms which are chemically identical with the normal atoms of the element in question, but are «labeled» by their radioactivity. The isotopes of Na and K used are quite unstable having a half-life of 15.5 and 13.5 hours, respectively, as found in our experiments. By the spontaneous splitting of the atom nuclei rays are emitted which can be utilized in a GEIGER-counter (HILDE LEVI 1941, I. HOLM-JENSEN 1943) to estimate the relative amounts of activity present in known volumes of solution. When an infinitesimal amount of, say,  $^{42}\text{K}$  is added to a certain volume of a bathing fluid containing, say, 1 mM of K we look upon the whole of the K present as labeled. A definite sample of it will give a definite count (decreasing of course with time at a certain rate), and when potassium penetrates into a cell placed in the fluid the counting of a sample of the sap will measure the amount of exchange with the potassium of the bathing fluid. When we have cells (containing, say, 100 mM K in the sap) which have been for a long time in a fluid of a certain composition (e. g. K content 1 mM) we assume a steady state to have been reached, so that in unit time just so much K is absorbed (mainly actively) from the bathing fluid as diffuses out from the sap of the cells. On this assumption it becomes possible by measuring the rate of uptake of  $^{42}\text{K}$  to estimate the rate of diffusion of K through the protoplasmic membranes and consequently the permeability of these.

### Material and methods.

The experiments were made on isolated cells from the stems of *Tolypellopsis stelligera* and *Nitella* sp. usually between 50 and 100 mm long with diameters of 0.6 — 0.9 mm in the case of *Tol.* and 0.5 — 0.7 mm in *Nitella*. Cells were placed either singly in paraffined tubes containing a small measured volume of experimental fluid or in groups in larger vessels containing so much that the changes in outside concentration could be disregarded. The containers were either stoppered or placed in a moist chamber, a box in which the air was kept saturated with water vapour. When a cell had to be analysed it was taken out of the experimental solution and usually washed thrice for one minute in each of 3 vessels with 50 ml of the same solution without activity. The turgor was ascertained, the diameter measured to 0.01 mm, adhering fluid removed, and the cell fixed by means of a lump of plasticine in a somewhat shorter glass tube so that a few mm of one end would protrude. In the later experiment these manipulations were carried out in a moist chamber into which the hands of the operator could be introduced through rubber cuffs, so that no fluid could be lost by evaporation. WARTIOVAARA had shown that the best way to obtain sap was to utilize centrifugal force. We accor-

dingly arranged a small hand centrifuge with a horizontal wooden bar on which the tube containing the cell could be clamped so that the free end of the cell at a distance of 14 cm from the axis was just inside the opening of a small sampling vessel. In this position the cell was either pricked by a fine needle (*Tol.*) or the end cut off (*Nit.*) and the sap driven off by centrifugation for a few seconds at about 300 revolutions/min. In the final series of experiments samples of protoplasm were next secured, after cutting off the end of the cell, in another sampling vessel by centrifugation for about 1 minute at about 1000/min. The sap obtained by these procedures is practically always free from chlorophyll, but the protoplasm contains an admixture of sap, and the outer layer containing most of the chloroplasts remains adhering to the cellulose wall. The quantities of sap and protoplasm secured were determined by weighing the sampling tubes. In almost all cases there would be enough sap from a single cell for a sample of about 10  $\mu$ l to be taken by means of a Carlsberg micropipette (MILTON LEVY 1936), placed in a counting dish and evaporated to dryness by exposure to radiating heat from an incandescent lamp as described by G. BILLMANN (1938 p. 10). The protoplasm, usually combined from two cells and amounting to 2–4  $\mu$ l from each, was transferred quantitatively by washing to a similar counting dish and likewise dried.

Representative samples of sap were analysed for potassium by the method described by KROGH (1943, 1944) and for total kations by conductivity determinations.

These determinations were carried out in a small vessel, containing 1 ml of distilled water. The sample to be analysed, usually about 5  $\mu$ l, was added and the conductivity between two platinated electrodes (each about 0.6 cm<sup>2</sup> with a distance of 0.8 cm) was measured by means of a Wheatstone-bridge arrangement (Philip Philoscope) using alternating current of 2 volts and 1000 Hz.

The conductivity was expressed by the concentration of KCl showing the same conductivity. From this figure the actual K concentration as found by the analysis was subtracted. The rest was corrected for Ca and Mg according to COLLANDERS analyses and recalculated as NaCl. This procedure is quite rough, but, as it turns out, sufficient for our purposes. We have assumed that the ionic composition of the protoplasm is identical with that of the sap and in a single case found it confirmed by analyses. As our supply of cells was rather small we could not do more. While the sap could be precipitated directly with platinum chloride the protoplasm of course had to be ashed in a platinum boat. Determinations accurate to about 2 % could be made on 10–20  $\mu$ l.

### The calculation of experiments.

We assume a concentration of the ion in question, the same in the sap of all the cells studied and remaining constant throughout the experimental period. A certain fraction, proportional to the concentration in mM ( $C_s$ ), the surface area (in  $\text{cm}^2$ ) and the time (hours), diffuses out through the protoplasmic membranes and is replaced by ions taken up (mainly by adenoid activity) from the bathing fluid. These ions are labeled by the radioactive isotope. By counting the activity of a unit volume of sap ( $a_s$ ) and of outside fluid

( $a_o$ ) we get the relation  $\frac{a_s}{a_o}$ .

In analogy with the earlier studies (COLLANDER 1933, WARTIOVAARA 1944) we have the permeation constant

$$k = 0.576 \frac{d}{t} \log_{10} \frac{C_s}{C_s - C_o \frac{a_s}{a_o}}$$

expressed in  $\text{cm}/\text{hour}$  and corresponding to the volume in  $\text{ml}$  of the inside solution diffusing out through  $1 \text{ sq. cm.}$  of the protoplasmic membranes in  $1 \text{ hour.}$

The activity of the protoplasm is in nearly all our determinations higher than in the sap, but a certain admixture of sap has to be allowed for. As shown below the cytoplasm volume of the cell in question can be calculated from a determination of the cell surface and the average thickness of the layer. This volume ( $\phi$ ) is subtracted from the actual volume of »protoplasm» obtained ( $P$ ) giving the sap mixed into it

$$\begin{aligned} P - \phi &= s \\ \text{and} \\ P \cdot a_P - s a_s &= \phi a_p \end{aligned}$$

from which the activity of the unmixed protoplasm ( $a_p$ ) is calculated.

When the activities of both sap and protoplasm are determined, we can, neglecting the diffusion resistance of the protoplasm itself, determine the diffusion resistance for each of the two membranes separating the protoplasm layer from the sap ( $R_i$ ) and the bathing fluid ( $R_o$ ), respectively.

The computation is carried out from the following differential equations.

$$-v_s \cdot \frac{dC_{si}}{dt} = k_i (C_{si} - C_{pi}) \quad (\text{I})$$

$$v_p \cdot \frac{dC_{pi}}{dt} = k_i (C_{si} - C_{pi}) - k_o \cdot C_{pi} \quad (\text{II})$$

in which  $v_s$  is the volume of the sap and  $v_p$  that of the protoplasm,  $C_{si}$  and  $C_{pi}$  represent the concentrations of the radioinactive ions in question in the

sap and protoplasm.  $k_i$  is the diffusion coefficient of the inner and  $k_o$  of the outer membrane.

For lim.  $v_p = 0$  we get from II

$$(II\ a) \quad k_i (C_{si} - C_{pi}) = k_o \cdot C_{pi} \text{ or } \frac{k_o}{k_i} = \frac{C_{si} - C_{pi}}{C_{pi}} \quad (II\ b)$$

In order to obtain an idea of the degree of approximation involved in II a we must solve equations I and II<sup>1</sup>.

Taking  $C_s = C_p$  we assume that  $C_{si}$  and  $C_{pi}$  may be expressed by the following series

$$C_{si} = C_s \sum \alpha_i \cdot e^{-\beta_i t}$$

$$C_{pi} = C_p \sum \delta_i \cdot e^{-\beta_i t}$$

By inserting these expressions for  $C_{si}$  and  $C_{pi}$  in (I) and (II) we get the following complete solution of (I) and (II)

$$C_{si} = C_s (a_1 \cdot e^{-\beta_1 t} + a_2 \cdot e^{-\beta_2 t}) \quad (III)$$

$$C_{pi} = C_s (\delta_1 \cdot e^{-\beta_1 t} + \delta_2 \cdot e^{-\beta_2 t}) \quad (IV)$$

where

$$\beta_1 = \frac{k_i \cdot v_s + k_i \cdot v_p + k_o \cdot v_s}{2v_s v_p} + \sqrt{\left( \frac{k_i v_p + k_i v_s + k_o v_s}{2v_s v_p} \right)^2 - \frac{4k_i \cdot k_o}{v_s v_p}}$$

$$\beta_2 = \frac{k_i \cdot v_s + k_i \cdot v_p + k_o \cdot v_s}{2v_s v_p} - \sqrt{\left( \frac{k_i v_s + k_i v_p + k_o v_s}{2v_s v_p} \right)^2 - \frac{4k_i k_o}{v_s v_p}}$$

$$a_1 = \frac{\beta_2}{\beta_2 - \beta_1} ; \quad a_2 = \frac{\beta_1}{\beta_1 - \beta_2} ;$$

$$\delta_1 = a_1 \left( 1 - \frac{k_i}{v_s} \cdot \beta_1 \right) \text{ and } \delta_2 = a_2 \left( 1 - \frac{k_i}{v_s} \cdot \beta_2 \right)$$

It is readily realized that with increasing  $t$ , we will reach a value  $T$  which will permit the links containing  $\beta_1$  in III and IV to be neglected.

For  $t \geq T$  we have

$$C_{si} = C_s \cdot a_2 \cdot e^{-\beta_2 t} \quad (V)$$

$$C_{pi} = C_s \cdot \delta_2 \cdot e^{-\beta_2 t} \quad (VI)$$

inserting these in II b we get

$$\frac{k_o}{k_i} = \frac{a_2 - \delta_2}{\delta_2} = \text{Konst.}$$

<sup>1</sup> We are greatly indebted to professor LINDERSTRÖM-LANG for suggesting to us the method of solution adopted. (Compare LINDERSTRÖM-LANG 1942).

It is readily realized from computations based on a few sets of arbitrary data similar to those found in our experiments, that the value of  $\frac{a_2 - \delta_2}{\delta_2}$  is practically equal to the real ratio  $\frac{k_o}{k_i}$ . In this way it is also seen that when  $t < T$  we will find  $\frac{k_o}{k_i}$  too small. In all our experiments  $T \leq 24$  h.

For application to our experiments (II b) is written

$$\frac{k_o}{k_i} = \frac{(C_s - C_o \frac{a_s}{a_o}) - (C_p - C_o \frac{a_p}{a_o})}{C_p - C_o \frac{a_p}{a_o}}$$

(because  $C_s = C_p$  must always be equal to the sum of inactive and active ions of the element studied).

$$\frac{k_o}{k_i} = \frac{C_o (\frac{a_p}{a_o} - \frac{a_s}{a_o})}{C_p - C_o \frac{a_p}{a_o}}$$

Since the last link of the denominator is in our experiments small compared with  $C_p$  we can use the convenient expression

$$\frac{k_o}{k_i} = \frac{C_o}{C_p} (\frac{a_p}{a_o} - \frac{a_s}{a_o})$$

As the resistance of the two membranes against the diffusion processes  $R_i$  and  $R_o$  may be expressed by  $\frac{1}{k_i}$  and  $\frac{1}{k_o}$  we have

$$\frac{R_i}{R_o} = \frac{k_o}{k_i} = \frac{C_o}{C_p} (\frac{a_p}{a_o} - \frac{a_s}{a_o}) \quad (\text{VII})$$

A small number of *Tolypellopsis* cells have been carefully measured as follows.

The length, exclusive of the ends with the attached small cells, directly on a mm scale. The diameter in micrometer units under the microscope and recalculated in mm. The cell was weighed in a tube. The sap driven off and weighed and thereupon the protoplasm. The cut ends were weighed and the cell wall dried and weighed. The results are given in table 1.

There is a satisfactory agreement between the volumes as calculated from the measured diameters and lengths and the weights. The sap obtained in this way is practically pure, and its quantity a fairly constant proportion of the total, but some sap remains and is driven off with the protoplasm, and



Table 1.

*Tolytellopsis* cells.

1	2	3	4	5	6	7		8
Length mm	Dia- meter mm	Vol. $\mu$ l.	Weight — ends mg	Sap mg	Proto- plasm mg	Cellulose wall		5/4 % Sap
						as dif- ference mg	dry mg	
80	0.895	50.7	50.5	38.4	6.4	5.7	1.7	76
75	0.74	32.4	31.5	24.0	4.2	3.3	1.2	76
73	0.762	38.6	39.3	31.6	4.0	3.7	1.2	81
57	0.733	24.2	24.0	16.6	4.2	3.2	1.0	69

some protoplasm containing the large majority of the chloroplasts remains adhering to the cell wall.

N. ANDRESEN, M. Sc. at the Carlsberg laboratory very kindly undertook some measurements on living cells both of *Tolytellopsis* and *Nitella* to determine the thickness of the protoplasmic layer, with the following results, extracted from his report.

The cells were examined through a water immersion lens magnifying 40 diameters and having a focal depth of  $2 \mu$ . The eyepiece magnified 15 times. Different impurities especially small algae were seen adhering to the surface. The thickness of the cellulose wall could not be ascertained. A fixed layer of chloroplasts was observed immediately below the adhering impurities. These had a horizontal diameter about  $10 \mu$  and a vertical height of  $4-6 \mu$ . The chloroplasts were so closely packed in *Tolytellopsis* that their circumference was polygonal.

The microscope was now focused on the under surface of the chloroplast layer and the position of the micrometer screw noted. Below this was a flowing cytoplasm filled with granules. In *Nitella* there were free chloroplasts and colourless granules of  $1-5 \mu$  diameter. In *Tolytellopsis* there were in addition bluegreen, more or less spherical bodies of  $5-10 \mu$  and flakes with a diameter up to  $50 \mu$  and about  $10 \mu$  thick. The microscope was lowered to the point when no more granules appeared by further lowering. In this position it was kept for about 5 minutes, and each time a larger indistinct granule passed the field of vision it was ascertained by raising and lowering the microscope whether it was above or below the focal plane adopted. If it was found below, the microscope was lowered accordingly and the final position noted.

This would give a maximum value for the cytoplasm thickness at the place examined. Such measurements were made near the ends and about the middle of each cell.

The variations in thickness observed within the single cells were small, and it was seen that the large inclusions in *Tolypellopsis* when followed over considerable distances would collide all the time with the fixed chloroplasts and fill up the cytoplasm to the lower boundary.

The measured thickness must be corrected for the focal depth, which is calculated and empirically measured to  $2\ \mu$ . At the highest position of the microscope this will be  $2\ \mu$  above the lower surface of the fixed chloroplasts, and at the lowest it will be  $2\ \mu$  below the lowest visible granule. The correction to be subtracted is therefore  $4\ \mu$ . As this happens to be also the thickness of the chloroplast layer we have accepted the figures as they stand as representing the total thickness of the protoplasmic layer.

The results are as follows.

Table 2.

<i>Nitella</i> .			<i>Tolypellopsis</i> .		
Cell I.	3 cm long.		Cell I.	2 cm long.	
	10 $\mu$	One end		15 $\mu$	
	10 »	Middle		13 »	
	10 »	Other end		15 »	
Cell II.	3 cm long.		Cell II.	3 cm long.	
	13 $\mu$			11 $\mu$	
	15 »			10 »	
	15 »			10 »	
Cell III.	6 cm long.		Cell III.	1.5 cm long.	
	12 $\mu$			15 $\mu$	
	10 »			18 »	
	10 »			15 »	
Cell IV.	2 cm long.		Cell IV.	1 cm long.	
	15 $\mu$			15 $\mu$	
	15 »			15 »	
	(25 » Very irregular scarcely reliable)			15 »	
Cell V.	3 cm long.		Cell V.	2 cm long.	
	15 $\mu$			12 $\mu$	
	14 »			13 »	
	15 »			12 »	

The average thickness for *Nitella* is  $13\ \mu$  and for *Tolypellopsis*  $13.5\ \mu$ .

COLLANDER (1930) puts the thickness of the protoplasm at  $5\ \mu$ , but his determinations were made on fixed material and on another species, *Chara ceratophylla*.

Utilizing the figure  $13.5 \mu$  for the *Tolypellopsis* cells in table 1 we calculate the protoplasm volumes given in table 3.

Table 3.

*Tolypellopsis* cells from table 1.

1	2	3	4	5	6	7	8
Length mm	Dia- meter mm	Surface cm <sup>2</sup>	Pl. vol. $\mu$ l.	Chloro- plast layer $\mu$ l.	Cytopl. $\mu$ l.	Sap corrected $\mu$ l.	% Sap
80	0.895	2.24	3.0	0.9	2.1	42.7	85
75	0.74	1.74	2.4	0.7	1.7	26.5	84
73	0.765	1.75	2.4	0.7	1.7	33.9	86
57	0.733	1.31	1.8	0.5	1.3	19.5	81

When the calculated cytoplasm volumes are subtracted from the plasma volumes given in table 1 the difference is sap and in column 7 this is added to the sap obtained by centrifugation.

We have analysed cell sap and protoplasm from 6 cells kept for about 11 weeks in 5 % ocean water containing 25 mM Na and 0.5 mM K. Sap was taken from each cell separately while the protoplasm from all was combined and ashed. K and conductivity were determined on each sample of sap. The results were:

Table 4.

Analysis of *Tolypellopsis* cells.

1	2	3	4	5	6
Cell	Sap mg	Protopl. mg	Kations <sup>1</sup> mM	K mM	Col. 4—Col. 5 mM
1	24.0	1.5	272	230	42
2	21.7	2.3	272	230	42
3	20.8	4.8	256	164	92
4	20.7	lost	258	200	58
5	16.9	2.4	lost	(178)	
6	17.0	5.9	286	177	109
Average:			269	200	69

<sup>1</sup> Expressed by the KCl concentration showing the same conductivity.

The 16.9 mg protoplasm collected contained 0.66 mg dry substance of which 0.33 mg was ash. The ash was dissolved in 2 ml water and determinations of K and conductivity made. Calculated as dissolved in 16.2 mg water the results would correspond to a conductivity of 252 mM with 174 mM K. It is evident that the assumption, which it is necessary to make in the permeability determinations, viz. that the composition of the sap is the same in all the cells examined and remains constant, represents only a first approximation.

In order to observe the changes taking place when cells were exposed to solutions of varying composition and the rate of such changes the following experiment was made. Solutions were prepared by mixing 200 ml of a 4 % dilution of seawater with suitable combinations of 60 mM NaCl and 60 mM KCl to obtain the following concentrations in mM.

Table 5.  
Experimental solutions.

	1	2	3	4
K .....	0.2	1.0	5.0	25
Na .....	39.7	39.0	35.2	15
Ca .....	0.22	0.22	0.22	0.22
Mg .....	1.13	1.13	1.13	1.13

The total kation content of these is the same, but Na is progressively replaced by K.

A number of cells from the sea water dilution mentioned above were distributed in these solutions on Decbr. 21 and samples taken out for analyses on 27/12, 10/1 and 24/1. Usually  $2 \times 2$  cells were taken each time, and 2 separate determinations made on the sap, while the protoplasm was combined from all 4 cells. Only two determinations on protoplasm could be made at each of the dates. In solutions 1 and 2 the cells kept well. In solutions 3 and 4 the cells showed increasing turgescence. On the 10/1 many had burst in solution 3, and only 1 was left alive in solution 4.

The average results are given in table 6.

Although the results are rather irregular there can be little doubt that in the solutions 1 and 2 the potassium concentration is gradually reduced while the total concentration is maintained. In the potassium rich solutions 3 and 4 potassium is taken up in excess and the total concentration rises, finally causing the cells to burst. It is to be remembered that the cells left for analysis are those which have shown the least reaction in this direction.

In a similar, but very incomplete, experiment begun early in Octb. a small number of cells were placed respectively in diluted sea water with a total

Table 6.

	Sap mM.			Protopl. mM.			
	Kation	K	Diff.	Kation	K	Diff.	
K 0.2	21/12	269	200	69	252	174	78
	27/12	266	175	91			
	10/1	248	155	93			
	24/1	252	143	109	229	112	117
K 1.0	21/12	269	200	69	252	174	78
	27/12	268	180	88	244	147	97
	10/1	250	161	89	185	99	86
	24/1	266	143	123	244	124	120
K 5	21/12	269	200	69	252	174	78
	27/12	284	218	66			
	10/1	301	197	104	220	137	83
K 25	21/12	269	200	69	252	174	78
	27/12	303	220	83	285	188	97
	10/1	341	226	115			

kation concentration of about 26 mM of which only 0.5 mM was K and in a portion of the same water to which KCl was added so as to raise the total concentration to 46 mM with 20 mM K. After 5 weeks the sap from a few cells was analysed for K and total kations with the following results.

Table 7.

	Na + K	K	Diff.
Low K	187	150	41
	195		
High K	197	188	7
	193		

In this case the sums of Na and K were obtained not from conductivity determinations, but directly according to the method of LINDERSTRÖM-LANG (1936). Lack of material prevented the repetition of this experiment which, if reliable, is interesting in showing a replacement of Na by K without any significant increase in total concentration.

It may be of interest that reactions similar to those now described were obtained by BOYLE and CONWAY (1940) in experiments on frog muscles. These, when exposed to increasing concentrations of potassium chloride from 12 mM up to 300 mM, while the external sodium remained constant at about 100, took up large amounts of potassium without swelling, but when the total

outside concentration was kept constant and sodium replaced with potassium, this ion was taken up with water causing a considerable swelling with constant potassium concentration inside the cells. It is shown by the authors that these results are predictable from the laws of physical chemistry and do not involve any active process. Observations made for us by dr. F. BUCHTHAL show that BOYLE and CONWAYS muscle cells had at all K concentrations above 12 mM irreversibly lost their vital properties. It is an interesting fact that in spite of this they would retain their very low permeabilities for a considerable period.

We have made a few observations showing that cells of *Tolypellopsis* and *Nitella* are able likewise to retain for hours a very low permeability after the flow of protoplasm has been brought to a standstill by heat (40—50° C) or a brief exposure to formaldehyde.

### Attempts to determine the uptake of activity by analysis of the bathing fluid.

In our first experiments with active preparations both of  $^{24}\text{Na}$  and  $^{42}\text{K}$  we tried to measure the uptake of activity from a small measured volume of active fluid. A cell was inclosed in a somewhat longer paraffined glass tube, 50 or 60  $\mu\text{l}$  solution were put in and the tube sealed with paraffin at both ends. When the tube was slowly rotated about a horizontal axis at right angles to its length the drop would move back and forth between the two ends of the cell. At suitable intervals samples of 5  $\mu\text{l}$  were drawn, dried and counted, and finally the cell was taken out, washed once by dipping into inactive solution and a sample of sap obtained by centrifugation. Activity disappeared rapidly from the external fluid which was reduced several per cent in less than 1 minute and generally about 20 % in an hour or so. As an example we give an experiment made on Novbr. 17 on a cell weighing 32.5 mg and placed in a paraffined tube in 50  $\mu\text{l}$  20 mM K with an activity put at 100. Samples of 4.8  $\mu\text{l}$  were taken at intervals with the results given in table 8. After 2½ hours the cell was taken out, superficially dried with filter paper and transferred to a second tube containing the same solution, but without activity. The general results of such experiments were very irregular and the final analyses of the sap showed only a minimal activity. We thought at the time that the results must be interpreted as a rapid uptake into the protoplasm, but it turned out that practically the whole activity lost from the external fluid was combined in the cell surface and largely in the cellulose wall.

This was borne out in a special experiment in which some cells of *Tolypellopsis* and *Nitella* were centrifuged so as to remove both sap and cytoplasm and leaving only the chloroplast layer and the cellulose walls. A cannula was tied into a few other cells and the content washed out completely with a current of salt solution.

The lengths of these skins were measured and they were exposed to a

Table 8.

1	2	3	4	5	6
Time	Bathing fluid		Total activity $2 \times 3$	Activity of sample	Activity disappeared
	Volume $\mu$ l	Activity			
10 <sup>50</sup>	50	100	5000		
10 <sup>53</sup>	45.2	80	3620	384	995
11 <sup>01</sup>	40.4	78	3150	364	
11 <sup>16</sup>	35.6	80	2850	384	
13 <sup>20</sup>	30.8	66.5	2050	319	415
13 <sup>22</sup>	50	0			
13 <sup>25</sup>	45.2	14.5	655	70	— 655
13 <sup>50</sup>	40.4	16.5	665	79	— 80
Sap	25.7	2.1	54		+ 621

solution containing <sup>24</sup>Na for 1 hour and for 2½ hours respectively. Thereupon they were taken out, washed in the usual way (3 × 1 minute), cut into pieces of suitable length, dried and exposed in the GEIGER counter. All of them showed activity, and in the case of *Tol.* with the layer of chloroplasts it amounted to 3—5 times the activity in a corresponding quantity of solution, while the activity of the cellulose membrane alone was not much in excess of (or definitely lower than) the solution which it might contain (but which one would expect to remove by the washing). In *Nitella* on the other hand most of the activity appeared to be located in the cellulose membrane. The experiments are, however, too few and the results too discordant to settle this point. They are only sufficient to show that activity determinations on the bathing fluid cannot give any reliable information regarding the uptake into the living cells, because large and variable amounts are «adsorbed» to the cell surface.

### Preliminary experiments with analysis of the cell sap.

In a preliminary experiment on Oct. 26 5 cells of *Tolypellopsis* were put into 25 mM labeled Na. The cells were taken out at intervals from 1.5 to about 10 hours and samples of the sap analysed for activity. In per cent of the bath-

ing fluid the activity amounted to 0.9 to 1.6, showing no increase with time and it was concluded that the activity found was mainly due to contamination with outside fluid as no washing was carried out.

In a second  $^{24}\text{Na}$  experiment, Novbr. 7—11, 5 cells were put into paraffined tubes, each charged with 60  $\mu\text{l}$  bathing fluid with 26 mM labeled Na, very nearly the same concentration in which they had been living for 6 weeks, and rotated as described for 83 hours. The concentration of Na in the sap is assumed to be uniform at 40 mM. Samples of 4.8  $\mu\text{l}$  were taken after 1, 36 and 83 hours and showed a somewhat irregular fall in activity. The sap was collected from each cell separately without washing and the activity determined.

The following activities were determined in % of the original bathing fluid.

Table 9.

1	2	3	4	5	6
Bathing fluid after		Sap after 83 hours		Sap corrected	$C_o \frac{a_s}{a_o}$
1 h.	83 h.	% of initial	% of final	$\frac{\text{col. 3} + 4}{2} - 1.4$	
91	82	7	8.5	6.3	1.63
95	110 <sup>1)</sup>	9	8.2	7.2	1.87
78	86	7	7.2	6.2	1.61
84	73	5	7	4.6	1.19
99	82	11	12.5	10.3	2.67

In column 5 is given the average percentage concentration of the sap, corrected for the contamination found in the experiment of Oct. 26.

Calculated according to the formula we find:

Table 10.

1	2	3	4	5	6
Diameter of cell $\text{cm} \times 10^{-2}$	$0.576 \frac{d}{t}$ $\times 10^{-4}$	$C_s - C_o \frac{a_s}{a_o}$	$\log (3)$	$\log C_s$ $-\log (3)$	col. 2 $\times$ 5 $K \times 10^5$
7.9	5.5	38.37	1.5840	0.0181	1.0
7.2	5.0	38.13	1.5812	0.0209	1.0
7.4	5.1	38.39	1.5842	0.0179	0.9
9.0	6.2	38.81	1.5889	0.0132	0.8
6.3	4.4	37.33	1.5721	0.0300	1.3
				Average	1.0

1) Sealing of tube incomplete. Some evaporation.



In determinations made on Novbr. 21—27 12 cells were placed in 25 ml 25 mM active sodium chloride, 3 cells were analysed separately after 106 hours and 8 more after 147—148 hours in groups of 2, 2 and 4. In this case the cells were carefully washed in the same solution without activity before taking the sample of sap.

The results after 106 hours were  $K$  values of 1.0, 5.5 and  $3.1 \times 10^{-5}$  and after 147—48 hours 2.2, 3.3 and  $3.6 \times 10^{-5}$  giving an average of 3.1. We are unable to explain the higher values observed in this case. No precautions were taken in these preliminary experiments to guard against evaporation, but the error hereby introduced can scarcely be significant.

On Novbr. 17 25 cells with an average  $K$  concentration in the sap of 188 mM, which had been kept since the beginning of Octbr. in diluted sea water containing 20 mM  $K$ , were placed in 50 ml of the same solution containing  $^{42}K$ , and in another vessel 25 cells taken from a solution with only 0.6 mM  $K$  and having an average  $K$  concentration in the sap of 150 mM were put into the same active solution. We expected to find a considerable difference in the uptake of activity, because the second group of cells had not reached a steady state with the bathing fluid. Cells were taken out at intervals, washed  $2 \times 3$  min. in inactive salt solution, dried rapidly with filter paper and the sap obtained by centrifugation without any protection against evaporation. The results are given in table 11.

Table 11.

Exp. with  $^{42}K$  on *Tol.* I  $C_s$  188,  $C_o$  20 mM  $K$ , II  $C_s$  150,  $C_o$  20 mM  $K$ .

I.									
Hours	2.8		5.5		15.1		28		81
$d \text{ cm} \times 10^2$	7.1	7.3	7.1	7.9	6.5	6.8	7.3	7.5	9.0 8.7
$C_s - C_o \frac{a_s}{a_o}$	176.4	178.3	165	178.3	147	161	161	180	82 52
$K \times 10^5$	(40)	(35)	42	19	28	18	10	2.9	23 34
II.									
Hours	2.6		5.3		15.0		28		80
$d \text{ cm} \times 10^2$	7.1		7.3	7.5	6.8	6.6	6.8		7.9 7.1
$C_s - C_o \frac{a_s}{a_o}$	144.2		199	127	98	140	102		0 0
$K \times 10^5$	(27)		79	58	48	7.5	23		

The average for all the cells in group I is  $25 \times 10^{-5}$  cm, and for the cells in group II  $40 \times 10^{-5}$  cm. When the determinations after 2.8 hours are left out, as less reliable, the values will be 21 and 43 respectively.

### Final experiments with determinations on sap and protoplasm.

These experiments were carried out late in February and with all the precautions we could think of, but it is possible that the *Tolypellopsis* cells, which had been kept in the laboratory for over 5 months, exposed to artificial light of low intensity, were not in perfect health. The turgor was good, however, and slow plasma rotation was observed, although with some difficulty, in a number of cells. The *Nitella* material was quite fresh and in excellent condition.

In tables 12 and 13 the results are given, and for the protoplasm we have included both the activity figures directly measured  $C_o \frac{a_P}{a_o}$  and those calculated for the unmixed protoplasm by means of the formula on p. 6  $C_o \frac{a_P}{a_o}$ .

Table 12.

Exp. with  $^{24}\text{K}$  on *Tol.*  $C_s = C_p$  150,  $C_o$  1.43 mM K and *Nitella*  $C_s = C_p$  90,  $C_o$  0.6 mM K.

Hours	<i>Tolypellopsis</i>						<i>Nitella</i>				
	1	6.7	20.7	23	44.8	68.8	1	5.8	19.7	43.8	67.9
$d \text{ cm} \times 10^2$	6.7	7.2	7.2	6.7	8.0	7.1	5.9	5.2	5.8	7.0	5.6
$C_o \frac{a_P}{a_o}$	0.40	0.50	8.9	9.0	3.7	6.1	0.84	1.74	2.5	5.0	2.3
$C_o \frac{a_P}{a_o}$	0.90	1.15	10.7	9.1	4.3	6.1	0.72	4.4	5.0	9.5	2.9
$C_o \frac{a_s}{a_o}$	0.04	0.11	8.0	8.7	3.4	6.1	0.17	0.27	1.26	2.0	1.6
$K \times 10^5$		(0.2)	4.8	4.4	1.0	1.1		(0.8)	1.0	0.9	0.4
$\frac{R_i}{R_o} \%$		(0.7)	1.8	0.3	0.6			(4.6)	4.2	8.5	1.4

It is perhaps worthy of note that a fairly large concentration of labeled ions is reached in the protoplasm within the first hour, suggesting the results obtained by BROOKS (1939) and MULLINS (1940) for active potassium and by AGNES WERNSTEDT (1944) for active lead.

It requires several hours before a steady state is reached allowing a calculation of  $K$ . Even so the results are rather discordant. This is not to be wondered at, because the method is indirect, and we cannot be sure that the diffusion out from the cell is at every moment exactly balanced by the uptake. Even larger discrepancies are seen when the different experiments are compared in table 14.

Table 13.

Exp. with  $^{24}\text{Na}$  Tol.  $C_s = C_p$  50 mM,  $C_o$  44 mM and Nit.  $C_s = C_p$  113,  $C_o$  4.9 mM.

	<i>Tolypellopsis</i>					<i>Nitella</i>		
Hours	1.5	47.5	95.7	120	120	1	47.3	95.3
$d \text{ cm} \times 10^2$	6.2	6.5	6.2	6.6	5.6	6.3	6.0	5.8
$C_o \frac{aP}{a_o}$	0.57	1.8	2.6	4.8	9.2	0.04	1.7	0.73
$C_o \frac{a_p}{a_o}$	1.6	2.2	3.2	7.5	16	0.073	2.6	0.7
$C_o \frac{a_s}{a_o}$	0.26	1.06	2.3	3.9	7.5	0.01	0.47	0.32
$K \times 10^5$		0.7	0.7	1	1.8		0.13	0.04
$\frac{R_i}{R_o}$		2.2	1.8	7	17		1.9	0.3

Table 14.

Summary of permeability determinations.

Potassium.							
	Date	Duration h.	Number of det.	$C_o$	$C_i$	$K \times 10^{-5}$ range      mean	
<i>Tolypel- lopsis</i>	17/11	15—81	6	20	188	2.9—28	— 18
	—	15—28	3	20	150	7.5—48	Cells from water with low potas- sium.
	23/2	21—69	4	1.4	150	1.0— 4.8	2.8
<i>Nitella</i>	—	20—68	3	0.6	90	0.4— 1.0	0.8
Sodium.							
<i>Tolypel- lopsis</i>	7/11	83	5	26	40	0.8—1.3	1.0
	21/11	106—147	6	25	40	1.1—5.5	3.1
	28/2	47—120	4	44	50	0.7—1.9	1.1
<i>Nitella</i>	—	47— 95	2	4.9	113	0.04—0.13	0.09

It seems certain that *Nitella* is less permeable than *Tolypellopsis*, as one would expect from the habitat in fresh water, often very poor in inorganic substances. It appears also as a fairly reliable result that potassium penetrates more easily than sodium, as one would expect from the relative dimensions of the two ions given by BOYLE and CONWAY as 1.49 for sodium, when potassium is taken as 1.00.

We ourselves place most reliance on the lowest figures for  $K$ , but it seems quite possible that the permeability can show wide variations. Our figures are of the same order of magnitude as the lowest found by COLLANDER and BÄRLUND (1933) for organic molecules like glucose, which are practically insoluble in lipoids. The technique available to COLLANDER and BÄRLUND did not allow any precise determination for these very slowly permeating substances, but only the statement that the constant is lower than  $3 \times 10^{-5}$ . Unfortunately we have no means of ascertaining the relative dimensions and rates of movement of the ions and molecules respectively. Although the constants found are extremely low they would be sufficient to allow a fairly rapid exchange of ions in animal cells of small dimensions, like red blood corpuscles and muscle fibres. A  $K$  of  $1 \times 10^{-5}$  cm corresponds in a *Tolypelopsis* cell of 0.7 mm diameter to a half saturation period of 1200 hours or 50 days, but in a muscle fibre of  $25 \mu$  diameter to 43 hours only, when we make the probable assumption that practically the whole of the resistance is located in the surface membrane of the fibre. For a red blood corpuscle of the usual dimensions ( $8 \mu$  diameter and  $2 \mu$  thickness) the volume works out as  $88 \mu^3$  and the surface as  $125 \mu^2$ . In such a corpuscle the permeability of  $1 \times 10^{-5}$  would correspond to a half saturation period of 4.9 h, and there is no doubt that the actual permeability is definitely lower.

### **The relative diffusion resistances of the two protoplasmic membranes.**

The inner protoplasmic membrane separates two fluids which are assumed to be identical with regard to the concentrations of dissolved ions, and which are at least very similar so far as Na and K are concerned, while the outer membrane separates the cell content from a bathing fluid of widely differing composition. It is therefore to be expected that the main resistance towards diffusion is located in the outer membrane, and this is what we have found; that of the inner making up from less than one to a few per cent of the total. There is no reason to assume the existence of any active transport mechanisms in the inner membrane, although we have no material to disprove it, but the uncertainty of the calculation of activities in the protoplasm proper seriously reduces the reliability of the single determinations. The main fact of its being many times more permeable to ions than the outer cannot be doubted however.

## Summary.

In the sap of the large cells of *Characeae* a number of ions are found in concentrations far exceeding those in the bathing fluid, and the total concentrations are much higher. Examples of such concentrations are given and the dependence of the inside on the outside concentrations studied in a few cases.

It is assumed that the maintenance of the large concentration differences for single ions are brought about as a »steady state», the amount diffusing out being regularly replaced by an active »adenoid» uptake of the ion in question.

On this assumption the rate of diffusion is measured through determination of the uptake of radioactively »labeled» isotopes of the same elements, and methods are described for such determinations, after isolation of samples of the sap and the protoplasm of the cells.

Formulas are derived for expressing by means of activity determinations the diffusion constant in cm/hour and the relative diffusion resistance of the inner and outer protoplasmic membranes.

Data are presented for the weight and dimensions of the different constituents of *Tolypellopsis* cells.

The thickness of the protoplasmic layer has been determined microscopically on living cells and is on an average  $13.5 \mu$  in *Tolypellopsis* and 13 in *Nitella*. The differences in concentration of  $\text{Na}^+$  and  $\text{K}^+$  found between sap and protoplasm are slight and inconstant, and it is assumed that the concentrations are identical.

Determinations of the change in activity of small volumes of bathing fluid cannot be utilized to study the uptake into cells, because considerable amounts are »adsorbed» on the outside.

Two series of determinations of the rate of uptake of  $^{42}\text{K}$  in *Tolypellopsis* have given rather variable results, but the most reliable point to a permeability constant of  $3 \times 10^{-5}$ . In several cells much higher figures are found, up to constants of  $5 \times 10^{-4}$ , while the corresponding figure for *Nitella* from a small number of determinations is  $0.8 \times 10^{-5}$ . Experiments with  $^{24}\text{Na}$  have given a constant about  $1 \times 10^{-5}$  for *Tolypellopsis* and  $1 \times 10^{-6}$  for *Nitella*. The variations observed are much smaller than with potassium. The significance of these figures is briefly discussed.

Comparative determinations of the concentration of activity in protoplasm and sap, respectively, show that the permeation resistance resides mainly in the outer protoplasmic membrane, while the inner is responsible for a few per cent at most.

## REFERENCES.

- BIILMANN, GERDA, 1938. Über den Blutjodspiegel. Diss. Kopenhagen.
- BOYLE, P. and CONWAY, E. J., 1941. Potassium accumulation in muscle and associated changes. *J. Physiol.* **100**, 1—63.
- BROOKS, S. C., 1938. The penetration of radioactive potassium chloride into living cells. *J. of cell. a. comp. physiol.* **11**, 247—252.
- COLLANDER, RUNAR, Permeabilitätsstudien an *Chara ceratophylla*.  
I. 1930. Die normale Zusammensetzung des Zellsaftes. *This journ.* **6**, 1—18.
- & H. BÄRLUND,  
II. 1933. Die Permeabilität für Nichtelektrolyte. *Ibid.* **11**, 1—114.  
III. 1939. Die Aufnahme und Abgabe von Kationen. *Protoplasma* **33**, 215—256.
- HOLM-JENSEN, IB, 1943. A Method of Correcting for Counting Losses in GEIGER-MÜLLER Counters with Special Reference to the GEIGER-MÜLLER Counter Arrangement Described by HILDE LEVI (1941). *Acta physiol. Scand.* **5**, 271—276.
- KROGH, AUGUST, 1943. The Exchange of Ions between Cells and Extracellular Fluid. I. The Uptake of Potassium into the Chorion Membrane from the Hens Egg. *Acta physiol. Scand.* **6**, 203—221.
- ANNA-LOUISE LINDBERG and BODIL SCHMIDT-NIELSEN, 1944. The Exchange of Ions between Cells and Extracellular Fluid. II. The Exchange of Potassium and Calcium between the Frog Heart Muscle and the Bathing Fluid. *Acta physiol. Scand.*
- LEVI, HILDE, 1941. A GEIGER Counter Arrangement Applied to Measurements of Radioactive Indicators in Biological Research. *Acta physiol. Scand.* **2**, 311—323.
- LEVY, MILTON, 1936. A Micro Kjeldahl Estimation. *C. rend. Lab. Carlsb. ser. Chim.* **21**, 101—110.
- LINDERSTRØM-LANG, K., 1936. Microestimation of alkalies in tissue. *C. rend. Lab. Carlsb. ser. Chim.* **21**, 111—122.
- 1942. Approximate solution of certain diffusion problems in liquid systems. *C. rend. Lab. Carlsb. ser. Chim.* **24**, 249—279.
- MULLINS LORIN, I., 1940. Radioactive Ion Distribution in Protoplasmic Granules. *Proc. Soc. exp. Biol. and Med.* **45**, 856—858.
- WARTIOVAARA, V., 1944. The permeability of *Tolypellopsis* cells for heavy water and methyl alcohol. *This journ.* **34**, 1—22.
- WERNSTEDT, AGNES, 1944. On the absorption of lead into the cells of *Nitella*. *This journ* **35**, 1—8.